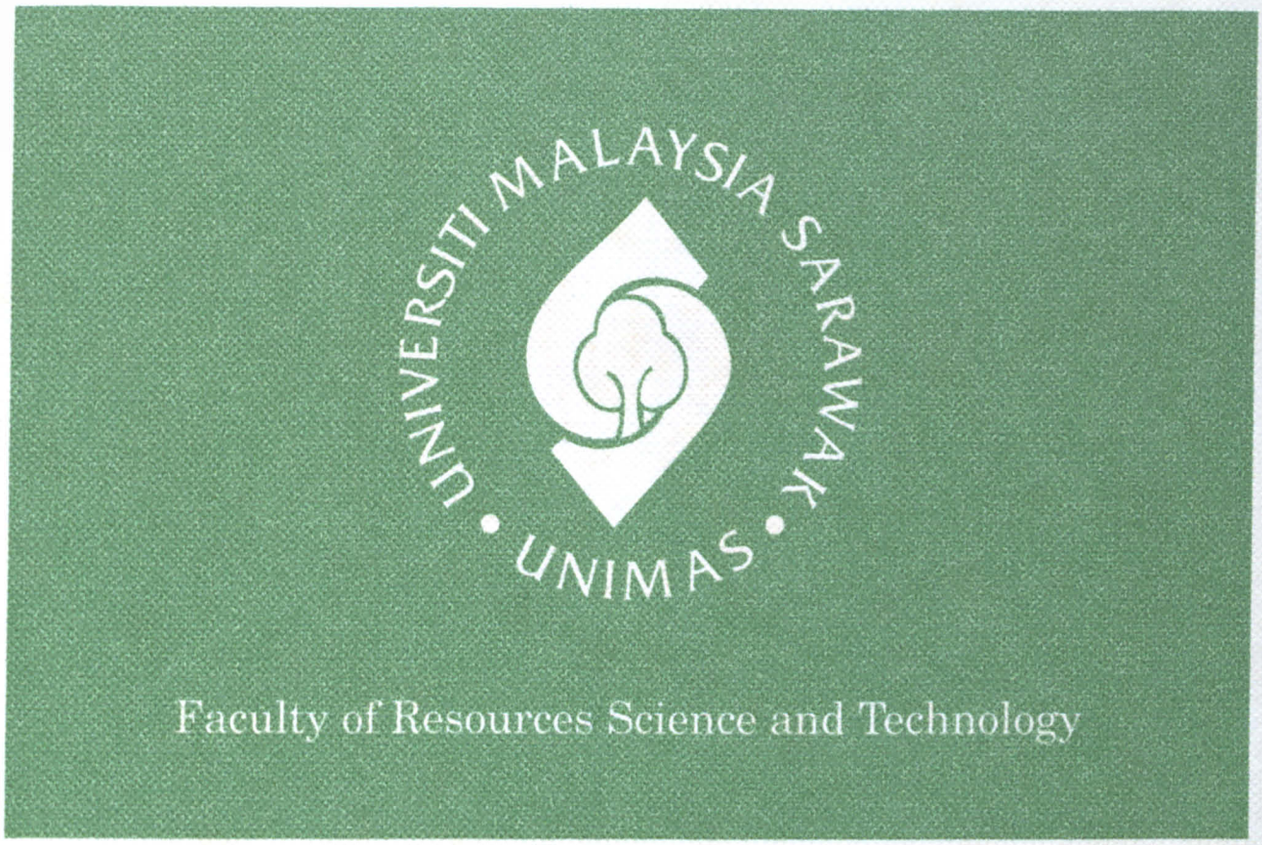

DIGITAL CONSTRUCTION AND COMPARISON OF HYPOTHETICAL THREE DIMENSIONAL PROTEIN MODELS OF HUMAN TUMOUR NECROSIS FACTOR (TNF) RECEPTOR-ASSOCIATED FACTOR (*TRAF*) GENES

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Digital construction and comparison of hypothetical three dimensional protein models of Human Tumour Necrosis Factor (TNF) Receptor-Associated Factor (*TRAF*) Genes

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List of Abbreviations

3D	Three-Dimension
ASK-1	Apoptosis Signal-regulating Kinase 1
BID	BCL-2 Interacting Domain
CASP	Critical Assessment of Structure Prediction
ERK	Extracellular signal-regulated kinase
FADD	Fas-associating protein with death domain
GCK	Germinal Center Kinase
GCKR	GCK-related
IL-1R/TLR	Interleukin-1 receptor/Toll-like receptor
jBID	JNK-mediated modification of Bid
JNK	c-jun N-terminal kinase
MAP	Mitogen-activated Protein
MAP3K	Mitogen-activated Protein Kinase Kinase Kinase
MATH	Merprin and TRAF-C Homology
MEKK1	MAPK/ ERK kinase kinase 1
NCBI	National center for Biotechnology Information
NF-κB	Nuclear Factor-kappaB
NIK	NF-κB-inducing kinase
NMR	Nuclear Magnetic Resonance
PrISM	Protein Informatics System for Modeling

PSI-BLAST	Position Sensitive Iterated-BLAST
RIP1	Receptor Interacting Protein 1
Smac	Second mitochondria-derived activator of caspase
TAK1	TGF-β Activated Kinase 1
TANK	TRAF-associated and NF-κB activator
TGF-β	Transforming Growth Factor-β
TNF	Tumour Necrosis Factor
TNFR	Tumour Necrosis Factor Receptor
TRADD	TNFR1 associated death domain
TRAF	TNF Receptor-Associated Factor
TβR	TGF-beta receptor
UniProtKB	Universal Protein Resource Knowledge Base

Digital construction and comparison of hypothetical three dimensional protein models of Human Tumour Necrosis Factor (TNF) Receptor-Associated Factor (*TRAF*) Genes

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ABSTRACT

TRAFs are the cytoplasmic adapter proteins that interact with the Tumour Necrosis Factor Receptor (TNFR) family. In human and mammals, there are seven members (TRAF1 – 7) in the TRAFs family. These members share a common TRAF domain at the Carboxyl terminus (C-terminus) and zinc fingers at the amino terminus (N- terminus) with the exception of TRAF1 that does not share the ring and zinc fingers, and TRAF7 that does not share the TRAF domain. Previous studies showed that TRAFs regulate the signaling or pathways which were closely related to the apoptosis process. Thus, these processes promote the survival of cancer cell and are linked to cancer development. In order to understand the function of TRAFs in cancer development and between TRAFs family member, three dimensional proteins (3D) models were constructed and used to simulate the interaction of TRAFs protein with other adapter protein in the pathway. The construction of TRAFs models were based on homology modeling, threading modeling and *ab initio* modeling methods using ModWeb, SWISS-MODEL and I-TASSER severs. There were total 77 models constructed for 7 TRAFs member from the 3 server; 18 from ModWeb, 24 from SWISS-MODEL, and 35 from I-TASSER. These models were then evaluated with ProSa-web, PROVE and PROCHECK tools. The full length models were used to perform docking simulation with ASK-1 and TRADD proteins.

Keywords: Tumor Necrosis Factor (TNF) Receptor Associated Factors (TRAFs), apoptosis, three dimensional (3D) proteins modeling, protein model evaluation, proteins docking simulation

ABSTRAK

TRAFs adalah protein-protein hubungan yang berinteraksi dengan keluarga "Tumour Necrosis Factor Receptor (TNFR)" dalam sitoplasma. Di dalam manusia dan mamalia, terdapat tujuh ahli (TAF1-7) di dalam keluarga TRAFs. Ahli-ahli ini mempunyai domain TRAF yang umum pada terminal karboksil (C-terminal) dan struktur "zinc fingers" pada terminal amino (N-terminal), kecuali TRAF1 dan TRAF7. TRFA1 tidak mempunyai struktur jari cincin dan zink, dan TRAF7 tidak mempunyai domain TRAF. Kajian-kajian dahulu menunjukkan TRAFs mengawal laluan biologi berkait rapat dengan proses apoptosis. Proses ini menningkatkan peluang hidup sel-sel kanser dan dikaitkan dengan perkembangan kanser-kanser. Untuk lebih memahami fungsi-fungsi TRAFs dalam perkembangan kanser, model tiga dimensi (3D) untuk TRAFs telah dibinakan dan diguna dalam menunjuk simulasi interaksi protein TRAFs dengan protein-protein dalam laluan biologi apoptosis. Model-model TRAFs telah dibina dengan kaedah " homology modeling" , "threading modeling" dan juga "ab initio modeling" dengan menguna ModWeb, SWISS-MODEL dan I-TASSER. 77 model telah dibinakan, 18 dari ModWeb, 24 dari SWISS-MODEL, dan 35 dari I-TASSER. Semua model telah dinilaikan dengan menguuna ProSa-web, PROVE and PROCHECK. Selepas itu, model-model yang lengkap telah digunakan dalam menunjuk simulasi interaksi protein TRAFs dengan protein ASK-1 dan protein TRADD.

Kata kunci: Tumor Necrosis Factor (TNF) Receptor Associated Factors (TRAFs), apoptosis, pemodelan tiga dimensi (3D) protein, penilaian model protein, simulasi interaksi protein

1.0 Introduction

Tumor Necrosis Factor (TNF) Receptor Associated Factors (TRAFs) are adapter proteins that interact with Tumour Necrosis Factor Receptor (TNFR) family such as TNFR1. TRAFs also interact with other protein such as Apoptosis Signal-regulating Kinase 1 (ASK-1), TNFR1 associated death domain (TRADD) and Fas-associating protein with death domain (FADD) to regulate the activation of transcription factor which mediate apoptosis process such as c-Jun N-terminal Kinase (JNK) and Nuclear Factor-kappa B (NF-κB) (Arch *et al.*, 2009; Liu *et al.*, 2000). The JNK and the NF-κB are crosstalk and inhibit the death of the cell (Lamb, *et al.*, 2003). When the apoptosis process in the cancer cell is inhibited, this will lead to cancer development.

Thus, the interaction between TRAFs with the receptors and proteins is important in the study of the function of TRAFs and lead to a better understanding of the apoptotic process in the cancer cell. The three-dimension (3D) models of TRAFs proteins are constructed based on homology modeling, threading modeling and *ab initio* modeling methods (Al-Lazikani *et al*, 2008;). The constructed TRAFs models are then used to perform docking simulation with other signaling protein, ASK-1 and TRADD (Fiser & Sali, 2003). Therefore, the objectives of this project are:

1. To construct the hypothetical three dimensional proteins (3D) models of Human TRAFs proteins with ModWeb, SWISS-MODEL and I-TASSER severs.
2. To evaluate the quality and possibility error occurred in TRAFs protein models with ProSA-web, PROVE and PROCHECK tools.
3. To analyze the interaction between TRAFs with signaling protein, ASK-1 and TRADD.

TRAFs protein sequences were retrieved from the Protein Data Bank (PDB) database and used to construct the 3D models. The TRAFs protein models were constructed using the ModWeb (Pieper *et al.*, 2008) Swiss-Model (Kopp & Schwede, 2004) and also I-TASSER (Zhang, 2007) modeling servers. The constructed TRAFs proteins models were evaluated using (Sippl, 1993; Winderstein & Sippl, 2007), PROCHECK (EMBL-EBI, 2010) and PROVE (Pontius et al, 1996; Marsden & Abagyan, 2003). After that, the TRAFs models were perform docking simulation using the Hex version 5.1 (Ritchie, 2008).

2.0 Literature Review

2.1 Tumour Necrosis Factor (TNF) Receptor-Associated Factor (TRAF)

2.1.1 TRAFs Family

TRAFs are the cytoplasmic adapter proteins that interact with the Tumour Necrosis Factor Receptor (TNFR) family. These proteins were first discovered in humans and rodents (Zapata *et al.*, 2007) and can also be found in other organism such as *Caenorhabditis elegans, Drosophila melanogaster*, and other mammals. In Human and mammals, there are seven members (TRAF1 – 7) in the TRAFs family and these members share a common TRAF domain at the Carboxyl terminus (C-terminus). This domain consists of about 180 amino acids that formed 7-8 anti parallel β-sheets fold (Henkler *et al,*2003; Zapata *et al.*, 2007). Besides the C-terminus, all the TRAFs, except of the TRAF1, contain ring and zinc fingers at the amino terminus (N- terminus). The TRAF7 is grouped with the TRAFs family although TRAF7 does not have the TRAF domain, but like other TRAFs, it contains the ring and zinc fingers at the N-terminus and it also seems to interact with the TNFR family (Zapata *et al.*, 2007).

2.1.2 Biological Function of mammalian TRAFs

According to Chung *et al.* (2002), the mammalian TRAFs mediate the signal transferring for the TNFR family and the interleukin-1 receptor/Toll-like receptor (IL-1R/TLR) superfamily. Besides that, TRAFs were found to react with a range of cell surface receptors which respond to stress and promote the cell survival through the regulation of the apoptosis process (Arch *et al.*, 1998). The TRAFs regulate the signaling or pathways such as JNK regulation, MAP activation, and NK-κB regulation (Chung *et*

al., 2002; Wajant *et al.*, 1998) which is closely related to the apoptosis process. Thus, these processes promote the survival of cancer cell and is linked to cancer development.

2.2 Nuclear Factor-kappa B

NF-κB was first discovered in the the nucleus of the B-cells. NF-κB is expressed in the cytoplasm of all cell types and translocated into nuclease when activated (Shishodia & Aggarwal, 2004). This protein is conserved from Drosophila up to humans. According to Shishodia and Aggarwal (2004), under different stimulus, cellular environment, or expression volume, the NF-κB can have duality function which basically mediates cell proliferation or cell apoptosis. The NF-κB proteins are grouped into 2 classes, where the class I proteins contain p50 (also known as NF-κB1), p52 (also known as NF-κB2), p100, and p105; and the class II proteins contain c-Rel, RelB, and RelA (also known as p65).

Activation of NF-κB can be stimulated or regulated by various agents such as cytokines, carcinogens, radiation and also TNF (Shishodia & Aggarwal, 2004). The TNF-induced NF-κB activation involves TRAFs proteins and basically through two pathways, which are canonical (classical) and non-canonical (alternative). In the canonical pathway, the activation is mostly mediated by TRAF2, TRAF5 and TRAF6; while in the non-canonical pathway, it is mostly mediated by TRAF2 and TRAF3 only. (Au &Yeh, 2007; He *et al*, 2007). For the TRAF1, it does not stimulate the NF-κB by itself, but together with the TRAF2 in the form of heterodimer (Lee & Choi, 2007). Kedinger and Rio (2007) pointed out that TRAF4 can increase NF-κB activation through a receptor called glucocorticoid-induced TNF-R (GITR).

2.3 c-Jun N-terminal Kinase

JNK (also called stress-activated protein kinase, SAPK) is a member of the Mitogen-activated protein kinase(MAPK) superfamily (Liu *et al.*, 2000; Yu *et al.*, 2004). JNK had been shown to play important role in many cellular activities such as growth control, tissue homeostasis, cell differentiation and programmed cell death (Chang and Karin, 2001, Yu *et al.*, 2004; Lamb *et al.*, 2003). JNK is activated by sequential protein phosphorylation through MAPK module and the mitogen-activated protein kinase kinase kinase (MAP3K) such as MAPK/extracellular signal-regulated kinase (ERK) kinase kinase 1 (MEKK1), ASK1 and transforming growth factor-β (TGF-β) activated kinase 1 (TAK1) which act as upstream activator for the JNK activation (Liu *et al.*, 2000; Yu *et al.*, 2004).

ASK1 does not only regulate JNK activation, but it also can activate p38 and promote apoptosis (Liu *et al.*, 2000). Liu *et al.* also stated that, in the activation of JNK, ASK1 interacts with TRAF2 and binds to TNFR1. Besides TRAF2, ASK1 can also interact with TRAF5 and TRAF6 in the JNK regulation (Liu *et al.*, 2000). Besides ASK1, TRAF 2 also can recruit the JNK though binding with germinal center kinase (GCK) and GCK-related (GCKR), and the MEKK1, or just with MEKK1 in the absence of coexpressed GCK/GCKR, but with the process of oligomerization of TRAF2 at receptor complex (Liu *et al.*, 2000). Yamashita *et al.* (2008) found that TRAF6 is required in the TGF-β induced activation of the JNK and p38. In the research, TRAF6 was shown to have the capability of binding to both TGF-β receptors, TβRI and TβRII, where the TRAF2 does not have this binding activity.

2.4 Collaboration of JNK with NF-κB and Caspase-8

In different cell types and under different stimulus, the JNK can be pro-apoptotic, anti-apoptotic, or does not involve in the apoptosis. Based on Lamb *et al.* (2003), the apoptosis are mediated by the activation of caspase-8 through recruitment of FADD to TNF, while for the anti-apoptosis, they are mediated by the activation of the NF-κB and Receptor Interacting Protein 1 (RIP1).

One of the apoptosis that is caused by the JNK will be the mitochondrial apoptosis pathway mediated by TNFα-Induced apoptosis (Lamb *et al.*, 2003; Deng *et al.*, 2003). The activation of the JNK induced the cleavage of pro-apoptotic molecule, BCL-2 Interacting Domain (BID) and the release of JNK-mediated modification of Bid (jBID). The jBID induces mitochondrial to release second mitochondria-derived activator of caspase (Smac), and the Smac will disrupt the TRAF2-cIAP complex in the TNFR1. When the complex is relieved from the cIAP inhibition, the caspase-8 are activated and apoptosis begins (Deng *et al.*, 2003; Yu *et al.*, 2004).

The TNFα-Induced apoptosis can be inhibited by NF-κB (Deng *et al.*, 2003). The JNK plays the anti-apoptotic role by mediating the activation of transcription factor, JunD, which can cooperate with NF-κB to increase the expression of pro-survival genes such as cIAP-2 (Lamb *et al.*, 2003).

2.5 Protein Structure Prediction and Modeling

Before the computational method available, all the protein structures are solved by using the experimental technique such as X-ray crystallography, nuclear magnetic resonance (NMR), and cryo-electron microscope, but these techniques are time

consuming and required protein identification, isolation, and purification (Buehler & Rashidi, 2005). According to Buehler and Rashidi (2005), solving the structure for new DNA and protein sequences daily cannot be done effectively using the experimental techniques. Therefore, the computational tools have to be introduced to solve this issue.

In the Critical Assessment of Structure Prediction (CASP), the protein structure prediction are grouped into 3 main methods, which are: 1) Comparative modeling (also known as Homology modeling), 2) Fold recognition (also known as Threading) and 3) Novel fold (also known as *ab initio*) (Al-Lazikani *et al.*, 2008; Lesk, 2008).

In homology modeling, the 3D protein model for the target protein (unknown structure) is constructed based on the structure of one or more similar or related template proteins (known structure) (Marti-Renom *et al.*, 2000). Based on Al-Lazikani *et al.*, (2008) and Sanchez and Sali (1997), the homology modeling has highest accuracy in constructing 3D structure hypothetical protein model compared to others. Fiser and Sali (2003) and Lesk, (2008) pointed out two important conditions that are needed in this technique, where the target and template proteins must have at least 30% similarity and the accuracy of the alignment between the target sequence and the template sequence.

Thread modeling or template-based modeling (unlike homology modeling), is for constructing many models based on known structures in the databases and all possible alignments between the target and template (Lesk, 2008; Xu *et al*, 2000). Besides that, Al-Lazikani *et al*, (2008) also pointed out that the templates identification method also different between this two template-based modeling categories; the homology modeling is based on target-template sequence similarity, while the threading is based on the target-

template structure compatibility. After that, the models build are evaluated and the best-scored model is selected (Lesk, 2008).

Unlike the methods discussed above, the *ab initio* method constructs protein structure based on the physical laws in the protein folding from the amino sequence of the target protein and also the physicochemical principles in the empirical energy functions (Al-Lazikani *et al*, 2008). This method is limited to short protein sequence, thus it connot be used to construct the whole protein model. It is more for the loop modeling.

The constructed protein models are useful in the studies of protein function, identifying active and binding sites, simulating protein-protein docking and others (Fiser & Sali, 2003; Sanchez & Sali, 2000).

2.6 Protein Modeling Tools

2.6.1 MODELLER and MODWEB

The MODELLER is one of the well known program that used homology modeling methods (Eswar *et al.*, 2003; Pieper *et al.*, 2008; Fiser & Sai, 2003; John & Sali, 2003). MODELLER is a freeware and can also be used for tasks such as alignment of two protein sequences, multiple alignment of protein sequence or structure, calculating the phylogenetic trees and loop modeling in protein structure (Eswar *et al.*, 2003; Fiser *et al.*, 2000).

MODELLER is a very useful tool in the proteins studies, but it uses Phyton scripting language to run the program, which is not user friendly for those who have no knowledge about scripting language (Sali & Blundell, 1993; John and Sali, 2003; Sali,

2009). Thus ModWeb has been introduced. ModWeb is a homology-modeling web server for MODELLER (Pieper *et al.*, 2008). To run on ModWeb, the input can be either insert of protein sequence or upload of FASTA file. Then, the tool will search through on the PDB databases for the suitable template. The ModWeb is fully automated for protein modeling only, thus less functions or tasks can be perform. To use ModWeb server, MODELLER license key is required (Pieper *et al.*, 2008)

2.6.2 SWISS-MODEL

SWISS-MODEL is another homology-modeling web server, which is fully automated (Kopp & Schwede, 2004; Arnold *et al.*, 2006; Schwede *et al.*, 2000; Schwede *et al.*, 2003). This server provides three main modes in file input for the protein model construction, 1) Automated mode, 2) Alignment mode, and 3) Project mode (Schwede *et al.*, 2003). SWISS-MODEL is been evaluated by the EVA-CM databases and provides high accuracy and reliability model output (Schwede *et al.*, 2003; Arnold *et al.*, 2006).

2.6.3 I-TASSER

I-TASSER web server provides an automated 3D protein structure prediction based on the I-TASSER algorithm, which is defined as Iterative, Treading, Assembly and Refinement (Zhang, 2007; Zhang, 2008; Wu *et al.*, 2007). Based on Zhang (2007), this algorithm is using the threading and *ab initio* methods to construct protein model and this consists of three steps, which are threading, fragment assembly, and iteration. This web server was ranked to be the best protein structure prediction in the CASP7 and CASP8 (Zhang, 2007; Zhang, 2009). The I-TASSER is freeware for all academic users.

3.0 Materials and Methods

3.1 Retrieval of TRAFs and Proteins Data

The sequences of all TRAFs were retrieved from the UniProtKB database in the FASTA format. Besides sequences of TRAFs, the identifier and data for proteins and receptors that interact with TRAFs were retrieved from the UniProtKB database (Table1) and PDB database.

Table 1: The identifier and amino acids lenght for each TRAFs member in the UniProtKB database

Human TRAF family member	Amino Acids length	Identifier
TRAF1	416	Q13077
TRAF2	501	Q12933
TRAF3	568	Q13114
TRAF4	470	Q9BUZ4
TRAF5	557	O00463
TRAF6	522	Q9Y4K3
TRAF7	670	Q6Q0C0

3.2 Construction of 3D structure Protein Model

3D structure protein models for TRAFs were constructed using MODWEB web server (Pieper *et al.*, 2008), SWISS-MODEL web server (Arnold *et al.*, 2006; Schwede *et al.*, 2003; Guex & Peitsch, 1997) and I-TASSER web server (Zhang, 2007; Zhang, 2008; Wu *et al.*, 2007). The models were constructed based on the amino acids sequences retrieved from UniProtKB.

For MODWEB, SWISS-MODEL and I-TASSER, the TRAFs sequences were inserted into the space provided and submitted to the web servers for modeling.

3.3 Evaluation of 3D structure Protein Model

The TRAFs structures constructed by the tools above were evaluated using ProSa-Web (Sippl, 1993; Winderstein & Sippl, 2007), PROCHECK (EMBL-EBI, 2010) and PROVE (Pontius et al, 1996; Marsden & Abagyan, 2003). For PROCHECK and PROVE, the evaluation were completed through the SAVes server.

3.4 Simulation of 3D structure Protein Model with Signaling Protein

The full length models of TRAFs from I-TASSSER were used to perform the simulation of protein-protein docking using the Hex version 5.1 (Ritchie, 2008). The docking simulation was performed between TRAFs models, the ASK-1 protein (PDB ID: 2CLQ) and the N-terminal domain of TRADD protein (PDB ID: 1F2H). The 3D structures of these two proteins were downloaded from the PDB database.

4.0 Results

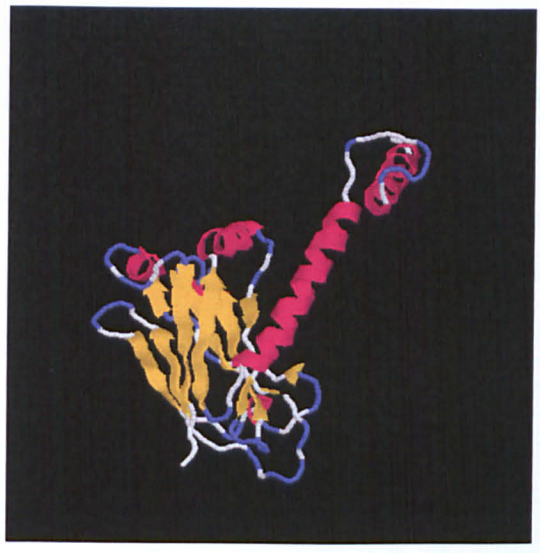
4.1 Constructed 3D structure Protein Model

All the models constructed were viewed in Rasmol version 2.6. In the MODWEB server, 18 models were constructed based on the seven TRAFs sequences. The summary about the models constructed are shown in Table 2.

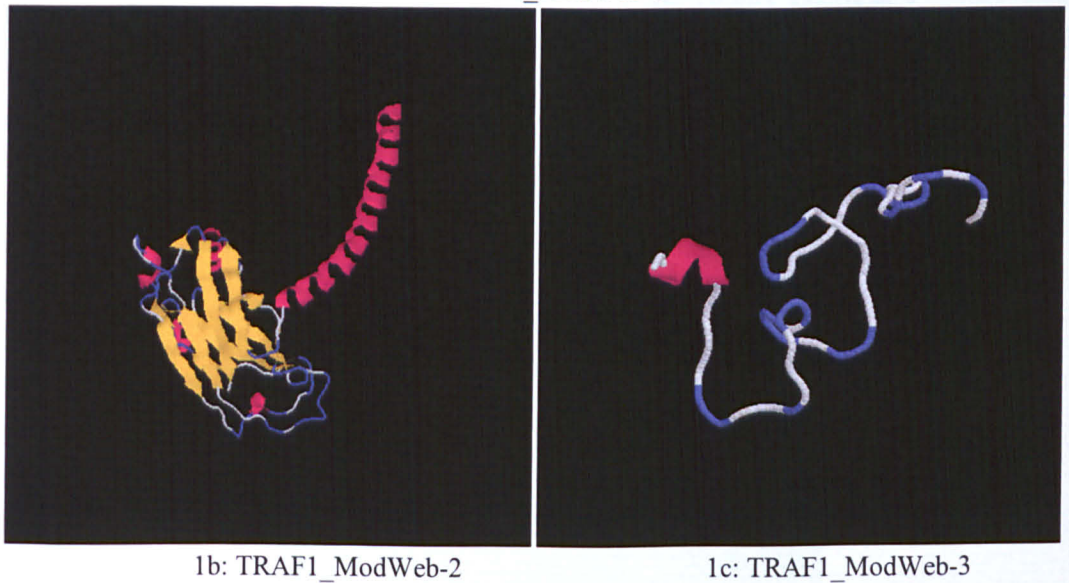
Table 2: Details about the Models constructed using the MODWEB server

Model	Length of the target sequence	Region of target sequence modeled	Template	Sequence identity of the alignment (%)
TRAF1_ModWeb-1	416	210 to 415	1flkA	52
TRAF1_ModWeb-2	416	226 to 413	1ca9A	57
TRAF1_ModWeb-3	416	4 to 60	1wesA	37
TRAF2_ModWeb-1	501	301 to 501	1flkA	48
TRAF2_ModWeb-2	501	311 to 501	1ca9A	100
TRAF3_ModWeb-1	568	364 to 568	1flkA	100
TRAF3_ModWeb-2	568	255 to 390	3i9yA	16
TRAF4_ModWeb-1	470	262 to 466	1flkA	36
TRAF4_ModWeb-2	470	185 to 248	2eodA	92
TRAF4_ModWeb-3	470	16 to 68	2bayA	25
TRAF5_ModWeb-1	557	349 to 556	1flkA	56
TRAF5_ModWeb-2	557	255 to 301	3basA	18
TRAF6_ModWeb-1	522	303 to 500	1ca9A	28
TRAF6_ModWeb-2	522	54 to 210	3hcsA	100
TRAF6_ModWeb-3	522	347 to 501	1lb6A	100
TRAF7_ModWeb-1	670	348 to 668	2pbiB	23
TRAF7_ModWeb-2	670	454 to 508	2aq5A	44
TRAF7_ModWeb-3	670	403 to 668	1p22A	30

All the models constructed by ModWeb server did not cover the whole sequences of the TRAFs retrieved. Most of the models of TRAF1 to 6 (Figure 1a, 1b, 2a, 2b, 3a, 4a, 5a, 6a, and 6c) showed TRAF domain composed of 2 four-stranded antiparallel β-sheets (shown in yellow colour) which linked by a few small α-helices (shown in red colour) and loops (shown in blue colour) , the domain was connected to a long α-helix (except for TRAF6_ModWeb-1 model in figure 6c) . Other models for TRAF1 to 6 are the coiled coil region (Figure 3b and 5b) and the zinc finger region (Figure 4b, 4c, and 6b). In the models of TRAF7 shown were the repeated regions, which composed by β-sheet structures. The TRAF7_ModWeb-2 model (Figure 7b) had shown the small part of the repeated regions. In the TRAF7_ModWeb-1 and TRAF7_ModWeb-3 models, the repeated regions are composed by 7 four-stranded antiparallel β-sheets linked by loops to form a circle structure. In the TRAF7_ModWeb-1, a α-helix structure is linked to the front part of the circle structure.

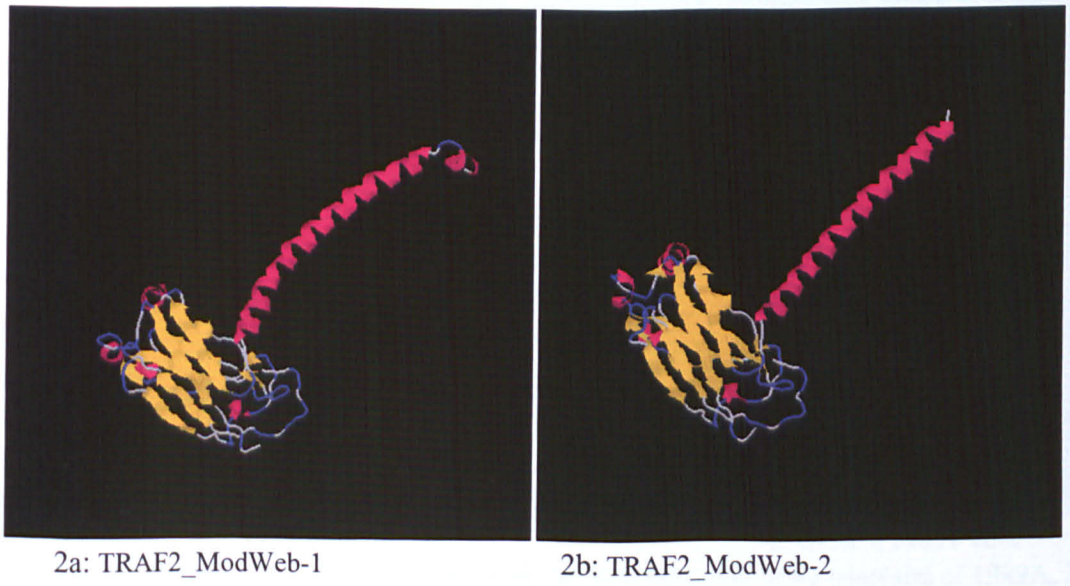


1a: TRAF1_ModWeb-1



1b: TRAF1_ModWeb-2 1c: TRAF1_ModWeb-3

Figure 1: The three TRAF1 models constructed by the MODWEB server using templates of 1flkA, 1ca9A, and 1weesA respectively



2a: TRAF2_ModWeb-1 2b: TRAF2_ModWeb-2

Figure 2: The two TRAF2 models constructed by the MODWEB server using templates of 1flkA and 1ca9A respectively